(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 January 2003 (23.01.2003)

PCT

(10) International Publication Number
WO 03/006494 A1

(51) International Patent Classification[7]: C07K 14/00, 7/06, A61K 38/08, 38/16, 47/42

(21) International Application Number: PCT/GB02/03212

(22) International Filing Date: 12 July 2002 (12.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0117011.7 12 July 2001 (12.07.2001) GB

(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF LEEDS** [GB/GB]; Leeds LS2 9JT (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **AGELLI, Amalia** [GR/GB]; Flat 9, Berkeley Court, Leeds LS2 9PT (GB). **BODEN, Neville** [GB/GB]; 77 High Ash Avenue, Leeds LS17 8RX (GB). **HUNTER, Malcolm** [GB/GB]; University of Leeds, Leeds LS2 9JT (GB). **KNOWLES, Peter** [GB/GB]; University of Leeds, Leeds LS2 9JT (GB).

(74) Agent: **HARRISON GODDARD FOOTE**; 31 St. Saviourgate, York YO1 8NQ (GB).

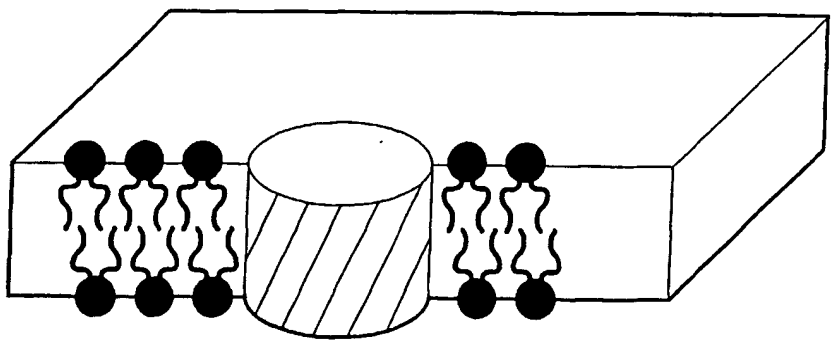
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PEPTIDE BETA-BARRELS



β-Barrel pores in lipid bilayers

(57) Abstract: There is described a self-assembling peptide beta barrel which comprises discrete peptide molecules each adopting a predominantly beta-strand conformation.

WO 03/006494 A1

PEPTIDE BETA-BARRELS

This invention relates to a novel form of beta-barrel pores made of self-assembling peptides, to methods of their production and to uses thereof.

It is known that some naturally occurring material polypeptides can exist in the form of beta barrels in vivo, for example porins and toxins appear as beta barrel structures embedded in cell membranes (Fig1).

Beta-barrels are cylindrical nanostructures made of the beta-sheet motif, with a hole in the middle. The beta-strand segments of the polypeptide chain which make the beta-barrel are proceeded and followed by other polypeptide segments which adopt turn, loops or helical conformations and are usually protruding outside the cell membrane. These intermediate segments also connect the beta-strand segments together. The long axes of the individual beta-strands making up the beta-barrel are parallel to or at an angle substantially less than 90° from the long axis the beta-barrel cylinder.

Porins, the major outer membrane proteins of Gram-negative bacteria, are responsible for the 'molecular sieve' properties of the outer membrane of Gram-negative bacteria. Porins consist of long polypeptide chains, each being several dozen to several hundred amino-acid residues long. Porins form large water-filled beta-barrel channels which cross the outer lipid membrane of bacteria, and which allow the diffusion of hydrophilic molecules into the periplasmic space. Porins also serve as receptor sites for the binding of phages and bacteriocins.

Toxins belong to a family of proteins/peptides which generally act by binding to membrane receptors. Toxins may cause death of the cells they attack. Toxins can consist of a large number of amino acid residues, for example, snake toxins may comprise sixty to seventy-five amino acids. A class of toxins are believed to be

characterised by their self-assembly into beta-sheet structures in the outer membranes of the cells they attack.

Thus, generally known beta barrel structures are made of long polypeptide chains or peptide molecules which are folded back upon themselves to form a barrel.

International Patent Application No WO 96/31528, Boden, et al describes peptides which self-assemble laterally in one dimension to form novel long beta-sheet tape-like polymers in a wide variety of different solvent conditions (Fig.2). Above a certain critical peptide concentration the tapes get topologically entangled and form a continuous three-dimensional gel network. These novel peptide gels possess the specific property of being able, under certain conditions, to switch from the gel state to a fluid or stiffer gel state.

We have now found that certain beta-sheet tape forming peptides can also give rise to beta barrels (Fig.3). In particular, by the appropriate selection of conditions and/or peptide structures, beta-sheet forming peptides can self assemble into beta barrel structures. Thus, we have found that, by the selection of peptides that exhibit desired hydrogen bond interactions and/or side chain interactions peptide beta barrels may be formed.

Thus, according to the invention we provide a self-assembling peptide beta barrel which comprises discrete peptide molecules each adopting a predominantly beta-strand conformation.

Moreover, we have found that peptide beta barrels can be triggered to disassemble by altering, for example, the pH. This action may be due to change of ionisation state of the amino acids in the peptide, the same charged species produced will tend to disassemble the barrel due to the repulsive like charges.

The self-assembling peptide beta barrels may be formed by using a lipid bilayer. The use of a lipid bilayer is able to influence the equilibrium between the peptide monomers and peptides in beta barrel structure. By selecting peptides which form beta sheets tapes which possess a hydrophobic width substantially the same as the thickness of the hydrophobic part of lipid bilayer, the peptide strands are able to hydrogen bond together to form a beta barrel which transverses the lipid bilayer. Such barrels will generally have a polar/hydrophilic core with an apolar/hydrophobic exterior.

According to a further feature of the invention we provide a method of preparation of a peptide beta barrel pore which comprises at least one of the following methods.

The beta barrels may be made by rationally designed peptides which self-assemble in the lipid membrane into beta barrels.

There are several alternative ways for the preparation of these beta barrels, examples of which include the following;

(i) A solution (preferably by aqueous solution) of self-assembling beta sheet forming peptides may be mixed with lipid bilayers.

The peptide concentration (c) in the solution can be low i.e. $c<c^*$ (where c^* is the critical peptide concentration for self-assembly in solution); in this case the peptides will be in the monomeric state. Alternatively if $c>c^*$, the peptides in solution will have self-assembled to form beta sheet polymers.

Upon interaction of the peptide solution with the lipid bilayers, a new equilibrium is established between peptides inserted in the bilayer and those in solution. In the bilayers, monomeric peptides may be helices, coils or beta strands. However, above a certain concentration c*barrel, the monomeric peptides spontaneously self-assemble into beta barrels.

(ii) Peptides in a self-assembled beta sheet state in solution can be switched to their monomeric state by external chemical triggering, e.g. pH change. The monomeric peptides thus generated can incorporate in a lipid bilayer, especially if the net charge of peptide is complementary to the net charge of lipid headgroups. Thus, electrostatic attraction between peptide and lipid, as well as hydrophobic interaction between peptide side-chains and lipid hydrocarbon chains, can favour formation of transmembrane self-assembled beta barrels.

(iii) A solution of peptide and lipid molecules in a common organic solvent can be prepared. The solvent may be removed by evaporation, and the dry peptide-lipid film produced may then be hydrated. In this way, lipid bilayers containing transmembrane beta barrel channel structures can be prepared.

The peptide beta barrels of the invention are advantageous, *inter alia*, because they can act as antimicrobial agents and bactericides, or they may be useful as drug delivery systems, as biosensors or as components in electronic devices.

The peptide beta barrels may function as antimicrobial agents or antibacterial agents and may act by forming a "hole" in the bacterium or microbe cell lipid bilayer.

As an antimicrobial agent the beta barrel peptides of the invention are especially useful in wound care. When bacteria grows in a wound site they lower the pH of the wound site. Thus if self assembling peptides are present (e.g. in a gel network state), this pH change can trigger some dissociation of the polymers to peptide monomers, which insert into the outer lipid bilayer of bacteria and form beta barrel holes. This will eventually lead to the death of the bacteria. Following elimination of the bacteria, the pH increases again to physiological values, and the peptides can reassemble, for example, into stable polymer fibrils in the wound site.

Alternatively, peptide polymers with such properties can be used to prepare antimicrobial wound dressing rather than gels which can be applied to wound site and be triggered to produce beta-barrel pore forming peptides in the cell membrane of the microbe.

Thus according to the invention we provide a wound dressing comprising a peptide as hereinbefore described.

The peptide beta-barrels can also be incorporated in the lipid bilayers of vesicles loaded with an active ingredient e.g. medicament/drug . This active compound can be small molecules, biologicals, proteonics, or DNA If these molecules can not penetrate the lipid bilayer, then the beta-barrels are the only means of release of the active compound outside the vesicle. Thus the beta-barrel in this case acts as a component of a formulation of slow or sustained release of an active compound. Of particular note, the self-assembly of the monomeric peptide into beta-barrel aggregates can be triggered in response changes to the pH if in an aqueous solution.

The peptide beta barrels may also have application in the oil industry. Specifically, our peptide beta barrels have potential application in both well construction (drilling, completion) and in reservoir stimulation (fracturing, water control). One particular application of beta-barrels in this field is the slow release of an active compound in the oil well. This can be done by incorporating the beta-barrel in lipid vesicles loaded with the appropriate active compound. The active compound will be released slowly though the beta-barrel. Increased well productivity resulting from reduced impairment of permeability in hydrocarbon-bearing formation would lead to fewer wells needing to be drilled to recover a given amount of oil. This represents significant cost savings when one considers that a typical horizontal well can cost up to £20 million. Improvements in reservoir productivity can have a dramatic impact on profitability and competitiveness. Current UK north sea oil production is about 1.6m barrels per day. At today's oil prices even a 1% increase in productivity would

yield an average revenue of £2,000 per day. The cost of effective treatments would therefore be rapidly recovered.

The peptide beta-barrels can allow ion flow and current to go through them. This can be detected using appropriate techniques (see Appendix). Their conductance properties can be altered by appropriate external triggers e.g. pH changes (see appendix). Thus beta-barrels can be used as components in sensor and electronic devices.

In yet a further preferred embodiment of the invention said beta-barrel material comprises peptides with hydrophilic or hydrophobic amino-acid residues or a mixture thereof and more preferably comprises an 4-40 residue peptide ideally a peptide 10-15 amino acid residues long.

The invention will now be described with reference to the accompanying examples and figures.

Figure legends

Figure 1:
Natural beta-barrels formed by porin-like proteins

Figure 2:
A beta-sheet tape formed by peptides which self-assemble in one dimension.

Figure 3:
A beta-barrel pore formed by self-assembling beta-forming peptides in a lipid bilayer. Each peptide is adopting a beta-strand conformation.

Example 1

Beta-barrel forming self-assembling peptides and their conductance properties

Peptide amino acid sequence using one-letter code:

DN1: QQ RFQWQFEQQ

DN1 –QF QQ RFQFQWQFEQQ

DN1-2E QQ RFEWEFEQQ

DN1-3OQ: QQ OFOWOFQQQ

The beta barrel channel-forming peptides are reconstituted into planar lipid bilayers by fusion of lipid vesicles containing the spanning channel.

The assessment of the conductance and of the assembly states of the transmembrane peptides is made by the planar lipid bilayer method, where the ion channel activity is studied under voltage clamp conditions.

Experimental conditions: pH 7-8, $R_{p:l}$= 1/500, 450mM KCl *cis* : 150mM KCl *trans*

The flux of ion through the channel is indicated by the fluctuations of current levels relatively to the baseline. They open and close in a stochastic way, reflecting the probabilistic nature the ion channel activity.

DN1 (Fig. 4): Regular ion channel activities, with square-top current fluctuations, long open dwell time, and low current amplitude (0.5 pA).

DN1-QF (Fig. 5): Regular and irregular ion channel activity, with square-top current fluctuation in certain moments as also with multiple levels of channel conductance in other moments, reflecting in this last case the presence of different association states for the peptides. It shows a varied range of current amplitudes (from pA up to hundreds of pA)

DN1-2E (Fig. 6a-c): Regular and irregular ion channel activity, with square-top current fluctuations in certain moments, as also erratic behaviour in other moments, including cluster of opening events, and low to high current amplitudes (0.5pA-10pA). On average the irregular activity is more frequent at higher voltages.

The remaining presence of irregular channel activity is due the not total neutralisation of the negative charges at this pH.

In this case, on average, we observe more regular ion channel activity when comparing (with the values of pH 7.5) for lower values of tension, but at higher tension an irregular ion channel activity is observed (however, on average, not so irregular as in the case of pH 7.5).

This indicate that the change of pH from 7.5 to 7 tend to neutralise more the negative charge of DN1-2E, and consequently produce more stable channel due the small peptide repulsion.

DN1-3ORN1Q (Fig. 7 a-c): This ion channel-forming peptide shows ion activity very irregular and with high amplitude current. This irregularity is possibly due the strong repulsion of peptides inside the bilayer that prevents the formation of a stable structure.

The ion channel activity at pH 8 still shows irregularity due the instability of the transmembrane structure, but this irregularity is less than those in the case of the pH 7.5, possibly due the diminish of the net positive charge.
At higher values of tension the high irregularity comes back.
At pH 8 the amplitude of current ranges from pA up to tenths of pA.

The occurrence of ion channel activity is much easier for DN1 and DN1-QF than to DN1-2E and DN1-3ORN1Q. In other words, it is much easier to observe the start of

current fluctuations, and the probability of open is bigger for DN1 and DN1-QF, than for DN1-2E and DN1-3ORN1Q.

This maybe due the instability of the aggregates constituted of charged peptides.

For DN1-2E at pH 8 and DN1-3ORN1Q at pH 7, the opposite behaviour is expected.

CLAIMS

1. A self-assembling peptide beta barrel which comprises discrete peptide molecules each adopting a predominantly beta-strand conformation.

2. A pharmaceutical composition comprising a medicament incorporated in a barrel of a self-assembling peptide beta barrel according to claim 1.

3. A pharmaceutical composition according to claim 2 characterised in that the composition is buffered such that the composition has a pH greater than 7.

6. A pharmaceutical composition according to claim 2 characterised in that the composition is suitable for the treatment of an enteral disorder and comprises therapeutically effective amount of an appropriate active medicament incorporated in a terminal hole of a β-sheet peptide tape.

7. A composition according to claim 4 characterised in that the composition comprises a therapeutically effective amount of an enterally active medicament in admixture with a peptide.

5. A method of delivering an enterally active medicament which comprises the administration of a composition which comprises a therapeutically effective amount of an appropriate active medicament incorporated in a barrel of a polypeptide beta barrel according to claim 1.

8. A method of wound healing which comprises administering a β-sheet peptide tape incorporating a medicament to a wound site.

9. A pharmaceutical composition according to claim 2 characterised in that the composition is suitable for use in wound care and comprises therapeutically effective

amount of an appropriate active medicament incorporated in a terminal hole of a β-sheet peptide tape.

10. A composition according to claim 4 characterised in that the composition comprises a therapeutically effective amount of a medicament suitable for use in wound care.

11. A composition according to either of claims 9 or 10 characterised in that the medicament is selected from one or more of the following; anti-puritics; antifungals, such as imidazole and triazole antifungals, e.g. clotrimazole, miconazole, econazole, sulconazole, chlorbutol, nystatin, ketoconazole, phenoxypropanol, natamycin, benzoyl peroxide and tolnaftate; antibiotics, such as tetracyclines and aminoglycosides, e.g. tetracycline hydrochloride, polynoxylin, chlortetracycline, mupirocin, neomycin, gentamycin and framycetin; antiseptics and disinfectants, e.g. triclosan, chlorhexidine, povidone-iodine, cetrimide, benzalkonium chloride, silver sulphadiazene, fusidic acid, hydrogen peroxide, polymyxin, hexachlorophane.

12. A wound dressing comprising fibrils or fibres which fibrils or fibres comprise two or more tapes twisted together characterised in that the fibrils or fibres have polypeptide beta barrel holes interspersed in them

13. A wound dressing according to claim 12 characterised in that one or more medicament is incorporated in at least some of the fibrils or fibres.

14. A method of wound healing which comprises applying a wound dressing according to claim 12.

15. A pharmaceutical composition according to claim 2 characterised in that the peptide is Lysβ-21.

16. A pharmaceutical composition according to claim 2 characterised in that the peptide comprises a hydrophilic peptide or a mixture of peptides.

17. A pharmaceutical composition according to claim 16 characterised in that the hydrophilic peptide comprises a 4-40 residue peptide or as mixture of such peptides.

18. A pharmaceutical composition according to claim 17 characterised in that the hydrophilic peptide comprises a peptide or a mixture of such peptides with a residue selected from 27, 24 or 21.

19. A pharmaceutical composition according to claim 18 characterised in that the hydrophilic peptide comprises the following 27 amino acid residues (KLEALYILMVLGFFGFFTLGIMLSYIR), or (KLEALYVLGFFGFFTLGIMLSYIR) for a 24 residue peptide.

20. A method of preparing a polypeptide β barrel according to claim 1 which comprises treating a β-sheet peptide tape at high pH.

21. A method of drug delivery which comprises:

(i) incorporating a medicament in a barrel of a polypeptide beta barrel according to claim 1.

(ii) administering the peptide incorporating the medicament; and

(iii) adjusting the pH to cause release of the medicament.

21. A method, a composition or a wound dressing substantially as described with reference to the accompanying description and examples.

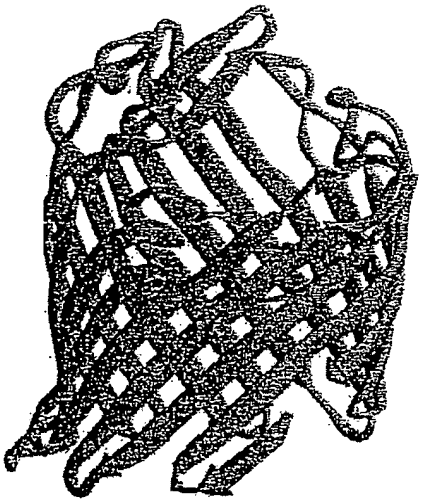


Fig. 1a

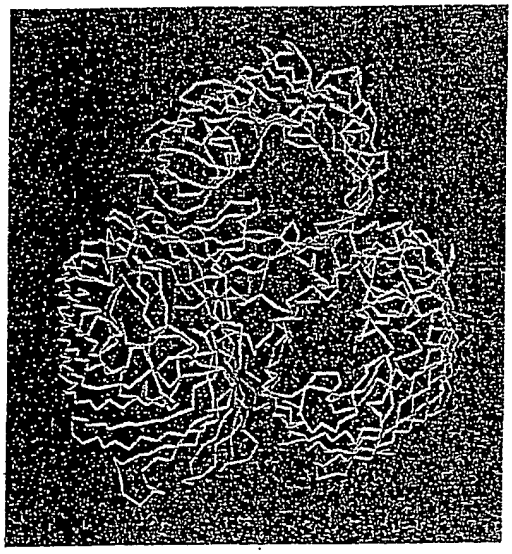
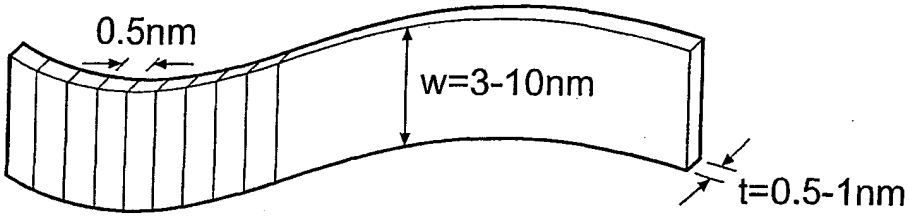
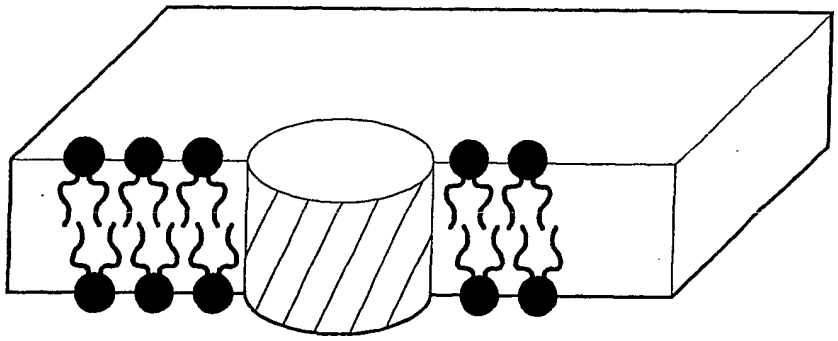


Fig. 1b



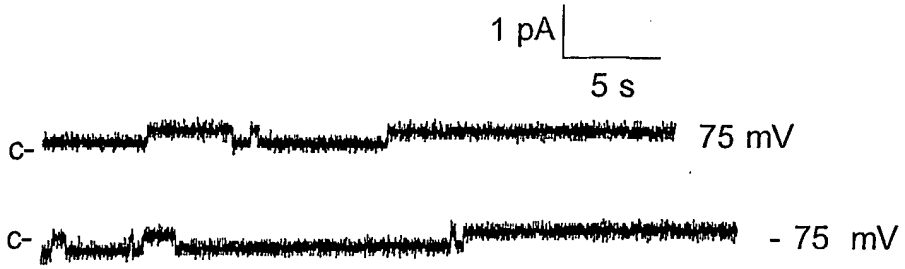
β-sheet tape

Fig. 2



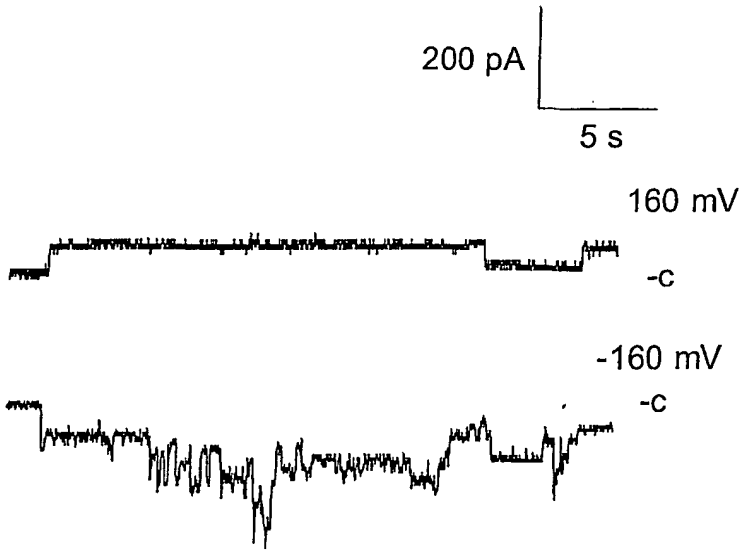
β-Barrel pores in lipid bilayers

Fig. 3



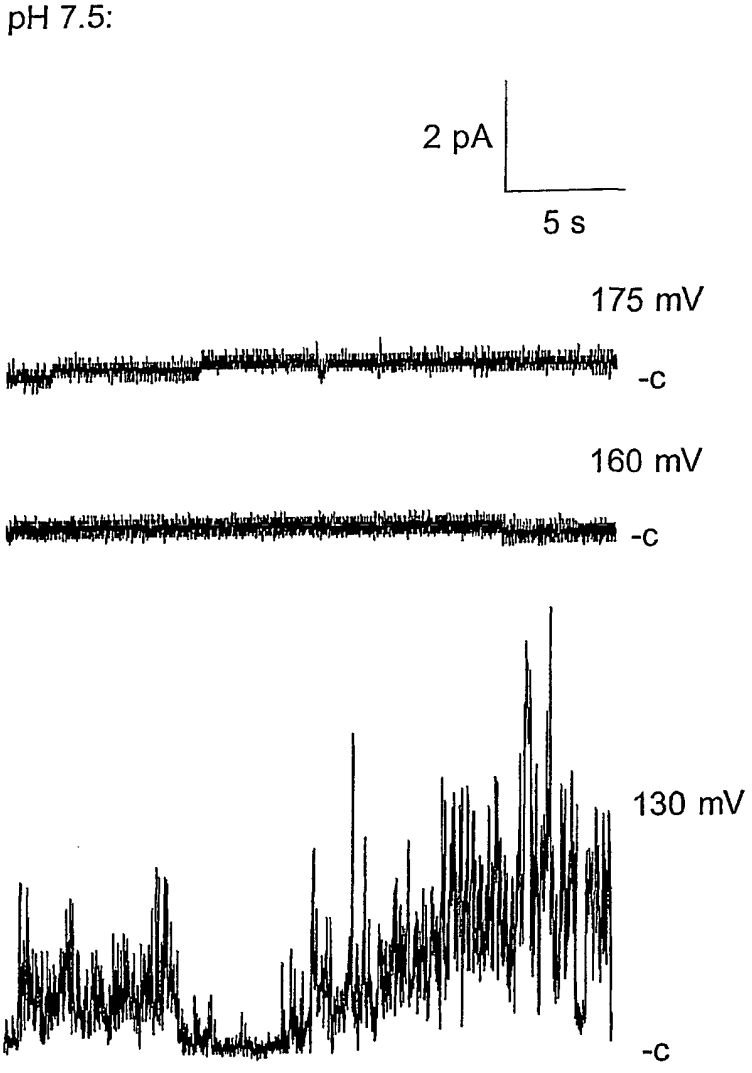
DN1: neutral charge, 11 amino-acid residues

Fig. 4



DN1-QF: neutral charge, 11 amino-acid residues

Fig. 5



DN1-2E: net negative charge (one positive and three negative charges), 11 amino-acid residues

Fig. 6a

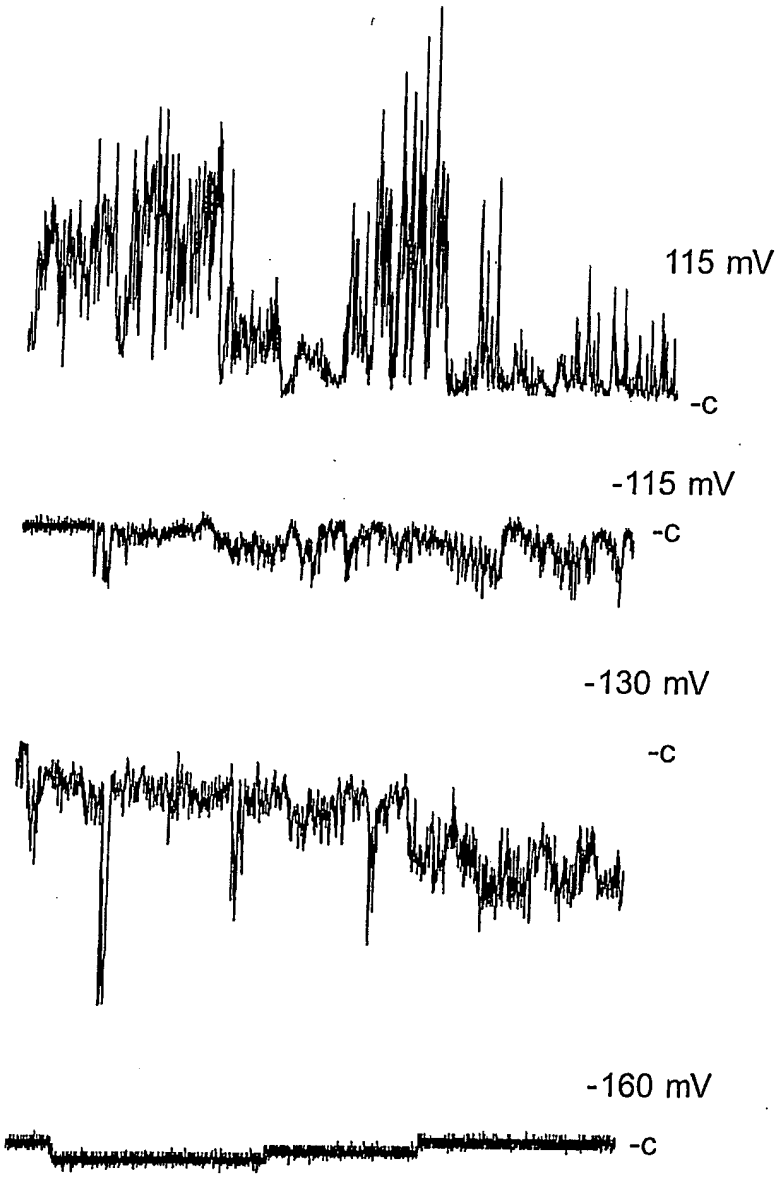


Fig. 6b

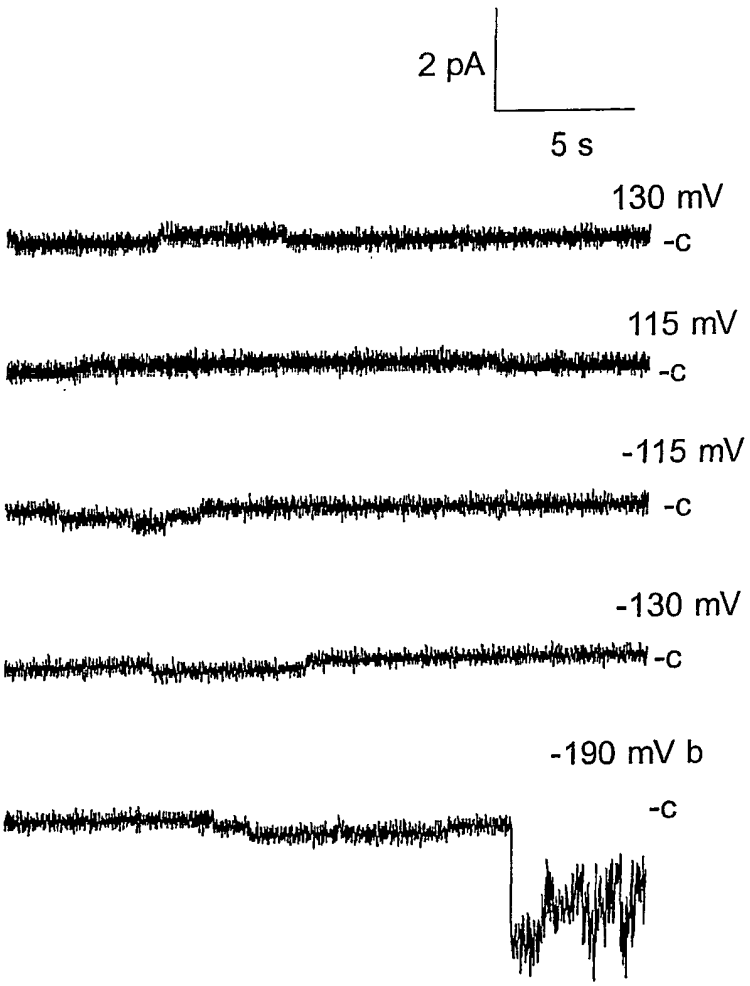
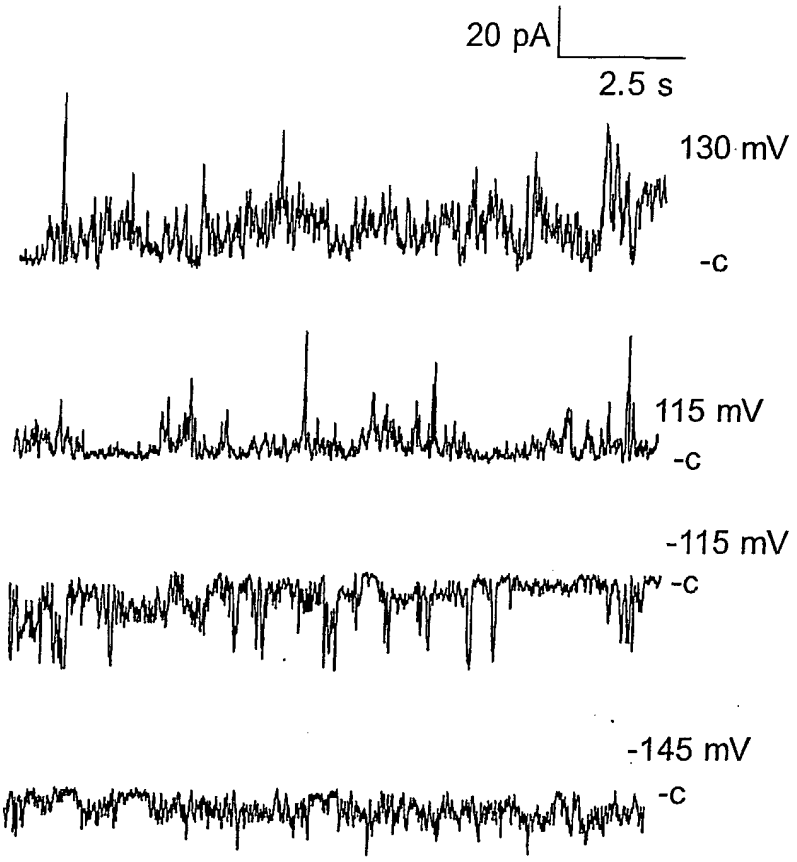


Fig. 6c



DN1-3ORN1Q: net positive charge (three positive charges), 11 amino-acid residues

Fig. 7a

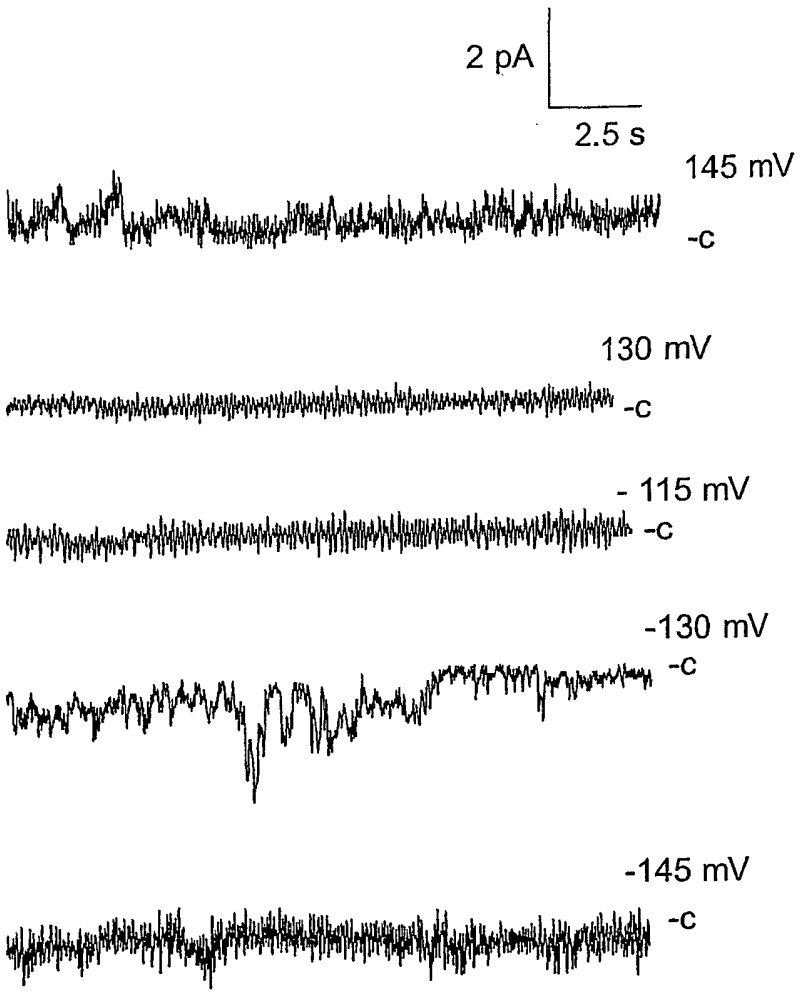


Fig. 7b

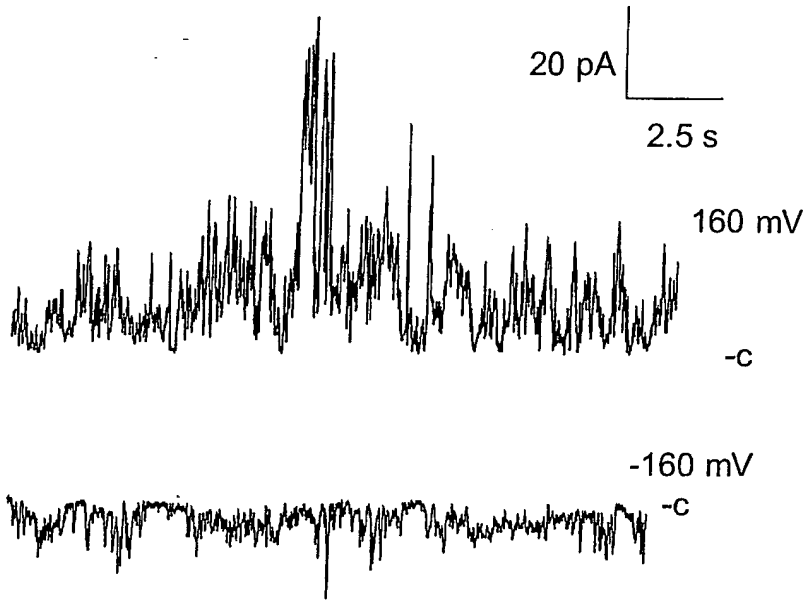


Fig. 7c

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/03212

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/00 C07K7/06 A61K38/08 A61K38/16 A61K47/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal, BIOSIS, PAJ, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHELEY STEPHEN ET AL: "Spontaneous oligomerization of a staphylococcal alpha-hemolysin conformationally constrained by removal of residues that form the transmembrane beta-barrel." PROTEIN ENGINEERING, vol. 10, no. 12, December 1997 (1997-12), pages 1433-1443, XP002218265 ISSN: 0269-2139 figures 1,2 --- -/—	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 October 2002	12/11/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ury, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/03212

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HORVATH LASZLO I ET AL: "Integration of a K+ Channel-Associated Peptide in a Lipid Bilayer: Conformation, Lipid-Protein Interactions, and Rotational Diffusion." BIOCHEMISTRY, vol. 34, no. 12, 1995, pages 3893-3898, XP002218266 ISSN: 0006-2960 abstract page 3898	1
Y	same passages	2,3,5,8, 16,17, 19-21
Y	WO 96 31528 A (UNIV LEEDS ;BODEN NEVILLE (GB); AGGELI AMAILIA (GB); MCLEISH THOMA) 10 October 1996 (1996-10-10) cited in the application whole document and in particular page 2 first paragraph and page 15, lines 17-22	2,3,5,8, 16,17, 19-21
X	page 3, line 17 - line 23	8
A	AGGELI, AMALIA ET AL: "Conformation and Ion-Channeling Activity of a 27-Residue Peptide Modeled on the Single-Transmembrane Segment of the IsK (minK) Protein" BIOCHEMISTRY (1998), 37(22), 8121-8131 , 1998, XP001117882 abstract	19
A	AGGELI, AMALIA ET AL: "Structure and Dynamics of Self-Assembling.beta.-Sheet Peptide Tapes by Dynamic Light Scattering" BIOMACROMOLECULES (2001), 2(2), 378-388 , 2001, XP001117883 abstract	19
A	NYRKOVA, I. A. ET AL: "Fibril stability in solutions of twisted.beta.-sheet peptides: a new kin of micellization in chiral systems" EUROPEAN PHYSICAL JOURNAL B: CONDENSED MATTER PHYSICS (2000), 17(3), 481-497 , XP001118807 abstract	
A	NYRKOVA, I. A. ET AL: "Self-assembly and structure transformations in living polymers forming fibrils" EUROPEAN PHYSICAL JOURNAL B: CONDENSED MATTER PHYSICS (2000), 17(3), 499-513 , XP001118808 abstract	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/03212

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 5, 8 and 21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 6, 7, 9, 10, 11, 12, 13, 14, 15, 18 and 21
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 02 /03212

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 6, 7, 9, 10, 11, 12, 13, 14, 15, 18 and 21

The following claims are so unclear that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

Claims 6, 9 and 11 (partially): a beta-sheet peptide tape does not have a terminal hole. No basis for beta-sheet peptide tapes having terminal holes can be found neither in the present description nor in the prior art.

Claims 7, 10 and 11 (partially): said claims refer back to a claim 4 which does not exist.

Claims 12, 13, 14: The formulation of claim 12 is totally unclear. Furthermore, said claim is not supported by the description.

Claims 15 and 18: The formulations "Lysbeta-21" and "a residue selected from 27, 24 or 21" are obscure even in the light of the description and the prior art.

Claim 21: see Article 17(2)(a)(ii) PCT and Rule 6.2(a) PCT.

Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely the remaining claims.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT
nformation on patent family members

In...onal Application No
PCT/GB 02/03212

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9631528 A	10-10-1996	AT	217323 T	15-05-2002
		AU	5155196 A	23-10-1996
		DE	69621085 D1	13-06-2002
		EP	0759933 A1	05-03-1997
		WO	9631528 A1	10-10-1996
		JP	10505106 T	19-05-1998
		NO	965142 A	30-01-1997
		US	2002132974 A1	19-09-2002